spontaneous calcium signaling of chondrocytes in short-term, but serum-free culture can better maintain calcium responses of chondroctyes in long-term culture. 3) Serum-free culture can significantly benefit the dynamic properties of cartilage compared with serum culture. These results imply that serum-free culture can maintain regular biochemical activities of chondrocytes better than serum medium, which may further benefit the biomechanical properties of cartilage explants.

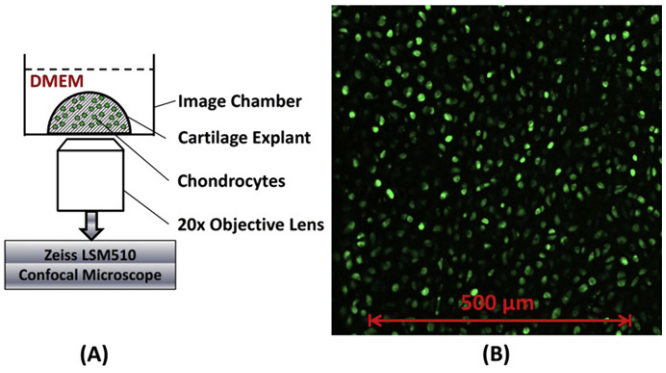

Fig. 1. (A) Cartilage explant on the confocal microscope; (B) A fluorescent image of chondrocytes in cartilage explant.

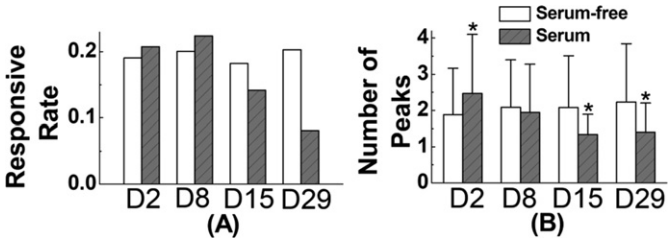

Fig. 2. Comparison of $[Ca^{2+}]_i$ responses between serum and serum-free group (serum-free group: 4068 cells, serum group: 3444 cells). (A) Responsive percentage of cells; (B) Number of peaks of responsive cells. (*: $p<0.05$, between the two groups).

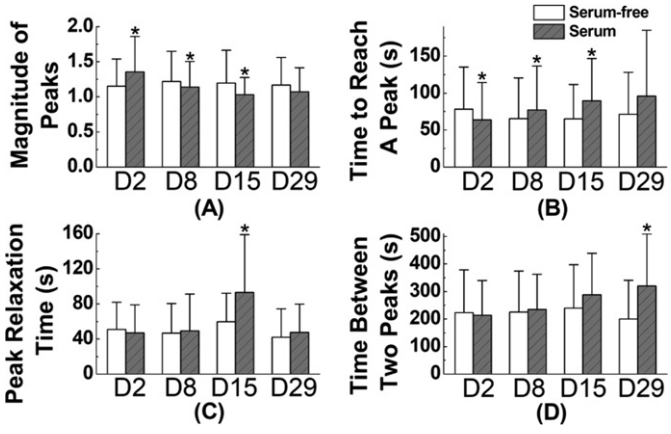

Fig. 3. Comparison of spatiotemporal properties of $[Ca^{2+}]_i$ responses. (A) Magnitude of all peaks for each cell (arbitrary unit); (B) Time to reach a calcium peak; (C) Peak relaxation time; (D) Time interval between two peaks. (*: $p<0.05$, between the two groups).

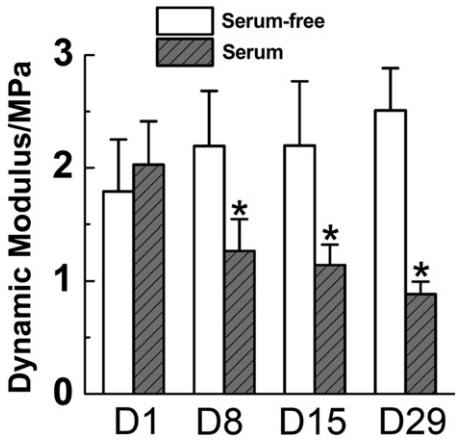

Fig. 4. Dynamic modulus of cartilage in serum and serum-free group (n=12). (*: $p<0.05$, between the two groups).

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ELEVATED HEPATOCYTE GROWTH FACTOR LEVELS IN OSTEOARTHRITIS OSTEOBLASTS CONTRIBUTE TO THEIR ALTERED RESPONSE TO BONE MORPHOGENETIC PROTEIN-2 AND REDUCED MINERALIZATION

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Purpose: Clinical and *in vitro* studies suggest that subchondral bone sclerosis due to abnormal osteoblasts (Ob) is involved in the progression and/or onset of osteoarthritis (OA). Human Ob isolated from sclerotic subchondral OA bone tissue show an altered phenotype, a decreased canonical Wnt/ß-catenin pathway (cWnt), and a reduced mineralization *in vitro* as *in vivo*. These alterations were linked with an abnormal response to BMP-2. OA Ob release factors such as the Hepatocyte Growth Factor (HGF) that contribute to cartilage loss whereas chondrocytes do not express HGF. A paracrine cross-talk between the subchondral bone compartment and articular cartilage may occur during OA via HGF. HGF can stimulate BMP-2 expression in human Ob, however, the role of HGF and its effect in OA Ob remains unknown. Here we investigated whether HGF in OA Ob is responsible for the altered response to BMP-2.

Methods: We prepared primary human subchondral osteoblasts using the sclerotic medial portion of the tibial plateaus of OA patients undergoing total knee arthroplasty, or from tibial plateaus of normal individuals at autopsy. The expression of HGF was evaluated by qRT-PCR and the protein production by Western blot analysis. HGF expression was reduced with siRNA technique whereas its activity was inhibited using the selective inhibitor PHA665752. Alkaline phosphatase activity (ALPase) and osteocalcin release (OC) were measured by substrate hydrolysis and EIA respectively. Canonical Wnt/β-catenin signaling (cWnt) was evaluated using two approaches: 1) target gene expression was measured using the TOPflash TCF/lef luciferase reporter assay, and 2) intracellular signaling partners β-catenin and phospho β-catenin levels were evaluated by Western blot analysis. Mineralization in response to Wnt3a was evaluated by Alizarin red staining.

Results: The expression of HGF was increased in OA Ob compared to normal Ob and OA Ob released more HGF as assessed by Western blot analysis. Transforming growth factor β-1 (TGF-β1) inhibited this expression in all cells. Conversely, HGF stimulated the expression of TGF-β1 when terminal differenciation of OA Ob was triggered with vitamin D_3. BMP-2 dose-dependently (1 to 100 ng/ml) stimulated both ALPase and OC in normal Ob whereas it inhibited them in OA Ob. Using HGF-siRNA treatments reversed this response in OA Ob and restored the BMP-2 response. cWnt was reduced in OA Ob compared to normal and HGF-siRNA treatments increased cWnt in OA Ob almost to normal. Smad 1/5/8 phosphorylation, which is reduced in OA Ob, was corrected with prior addition of PHA665752 to these cells. The BMP-2-dependent

mineralization of OA Ob which is also reduced compared to normal was partially restored by HGF-siRNA treatment whereas the addition of HGF to normal Ob reduced their mineralization.
Conclusions: OA Ob expressed more HGF than normal Ob. Increased HGF stimulates the expression of TGF-β1 in OA Ob and reduces their response to BMP-2. Inhibiting HGF expression or signaling restored the response to BMP-2 and Smad 1/5/8 signaling. In addition, increased HGF prevents the normal mineralization of OA Ob. In summary, this study raises the hypothesis that increased HGF levels may be implicated in the OA.

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HYPERTROPHIC DIFFERENTIATION DURING CHONDROGENIC DIFFERENTIATION OF PROGENITOR CELLS IS STIMULATED BY BMP-2 BUT SUPPRESSED BY BMP-7

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Purpose: Bone morphogenic protein (BMP)-2 and BMP-7 are clinically approved and their recombinant proteins are used for bone tissue regenerative purposes and widely evaluated in the context of cartilage regeneration. A comparison of the in vitro chondrogenic characteristics of BMP-2 versus BMP-7 on chondrogenic differentiation of progenitor cells and potential differentially induced downstream signalling has not been reported and was topic of investigation in this study.
Methods: Equimolar concentrations of BMP-2 or BMP-7 were added to chondrogenic differentiating ATDC5 or human bone marrow stem cells and samples were harvested at appropriate time points in chondrogenic differentiation. Expression of Col2a1, Sox9, Acan, Col10a1, Runx2, ALP, Mmp13, Mef2c and Bapx1/Nkx3.2 was determined by RT-qPCR and immunoblotting. Glycosaminoglycan (GAG) content and cell proliferation capacity were analysed by colorimetric analyses. The role of Bapx1/Nkx3.2 during chondrogenic differentiation under BMP conditions was investigated by targeting Bapx1/Nkx3.2 expression using a target specific siRNA duplex.
Results: BMP-2 dose-dependently increased expression of chondrocyte hypertrophic genes Col10a1, Runx2, ALP, Mef2c and Mmp13 during chondrogenic differentiation of progenitor cells. In contrast, the presence of BMP-7 during chondrogenic differentiating of ATDC5 cells resulted in decreased expression of chondrocyte hypertrophic genes and an overall increased Col2a1, Acan and Sox9 expression. Both BMPs did not influence the rate of cell proliferation, while both indistinguishably induced total GAG content. This profound differential action of BMP-2 and BMP-7 on ATDC5 chondrogenic differentiation could also be confirmed in hBMSCs. In a candidate approach Bapx1/Nkx3.2 was found to be involved in the BMP-7-mediated suppression of chondrocyte hypertrophy. As opposed to BMP-2, Bapx1/Nkx3.2 expression was specifically increased in the BMP-7 conditions while knockdown of Bapx1/Nkx3.2 expression abrogated the BMP-7-mediated hypertrophic suppression.
Conclusion: BMP-2 and BMP-7 display opposing actions on the chondrogenic outcome of differentiating progenitor cells: BMP-2 acts a specific inducer of chondrocyte hypertrophy, while BMP-7 appears to increase or maintain chondrogenic potential and suppress chondrocyte hypertrophy. Studies into the underlying mechanism revealed Bapx1/Nkx3.2 as a potential transcriptional mediator in the BMP-7-induced hypertrophic suppression. Based on these results we expect that differential use of BMP-2 or BMP-7 will benefit bone and cartilage regenerative techniques by inducing or preventing chondrocyte hypertrophy, respectively.

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HYALURONAN INHIBITS NUCLEAR FACTOR-κB ACTIVATION BY TYPE II COLLAGEN PEPTIDE IN OSTEOARTHRITIC CHONDROCYTES VIA CD44 AND ICAM-1

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Background: In addition to the proinflammatory cytokines, there is an increasing body of evidence that degradation products of cartilage matrix are important amplifiers or catabolic players in diseased joints like osteoarthritis (OA). Excessive degradation of cartilage matrix in OA involves enhanced cleavage of type II collagen by collagenases, especially matrix metalloproteinase (MMP)-13, resulting in denaturation of the triple helix of this collagen. Denatured and degraded type II collagen leads to an increase in proteolytic products of type II collagen. Recently, we have found that a 24-mer synthetic peptide of type II collagen named CB12-II stimulates type II collagen cleavage with induction of MMP-13 in cartilage explant culture. Although the intracellular signaling that leads to cartilage destruction is mediated by a cluster of catabolic pathways including nuclear factor-κB (NF-κB), the effect of CB12-II on NF-κB remains unclear. Hyaluronan (HA) of high molecular weight is widely used in the treatment of OA by intra-articular injection. An increasing body of evidence indicates that HA suppresses catabolic actions by proinflammatory cytokines like interleukin-1 and matrikines such as fibronectin fragments. However, little is known of HA effect on actions of CB12-II through interaction with HA receptors such as CD44 and intercellular adhesion molecule-1 (ICAM-1).
Purpose: This study was aimed to examine NF-κB activation in association with MMP-13 production by CB12-II and its inhibition by HA via its receptors, CD44 and ICAM-1 in OA chondrocytes.
Methods: With or without pretreatment with either 2700 kDa HA or BAY11-7085, cartilage explants harvested from OA knee joints or isolated chondrocytes in monolayer were incubated with CB12-II or the scramble peptide. In another set of experiments, following pre-incubation with anti-ICAM-1, anti-CD44, a combination of both antibodies, or non-specific IgG, cartilage explants harvested from OA knee joints or isolated chondrocytes in monolayer were incubated with or without 2700 kDa HA, followed by coincubation with CB12-II. Control cultures had no additives.
Enzyme-linked immunosorbent assays for phosphorylated p65 NF-κB and MMP13 were performed using total cell lysates and culture supernatants, respectively.
Results: When cartilage explants or chondrocytes in monolayer were incubated with CB12-II, the type II collagen peptide activated NF-κB in association with enhanced MMP-13 production. Inhibition studies with the specific inhibitor indicated the requirement of NF-κB for CB12-II-induced MMP-13 production. Pretreatment with HA resulted in significant suppression of CB12-II-stimulated MMP-13 production in cartilage as well as in chondrocyte monolayer cultures. HA suppressed NF-κB activation by CB12-II, leading to a decrease in MMP-13 production. While the individual antibody to CD44 or ICAM-1 partially reversed HA effect on CB12-II action, both antibodies in combination completely blocked the HA effect.
Conclusions: Administration of HA into osteoarthritic joints could suppress the catabolic action of matrix degradation products like CB12-II as a potent NF-κB inhibitor. The present study clearly demonstrated that HA suppressed CB12-II-activated NF-κB via CD44 and ICAM-1 in OA articular chondrocytes, leading to decreased MMP-13 production. Administration of HA into osteoarthritic joints could suppress the catabolic action of matrix degradation products like type II collagen fragments in OA joints through the mechanism demonstrated in this study.

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PROSTAGLANDINS AND CYCLOOXYGENASES IN CHONDROGENIC DIFFERENTIATION OF PROGENITOR CELLS

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Purpose: Prostaglandins (PGs) and their synthesizing enzymes Cyclooxygenase (COX)-1 and -2 are key factors in inflammatory processes, as well as in the biology of osteocytes and osteoclasts. Although known to be involved in the biology of the bony part of the skeleton, an involvement of PGs and COX-enzymes in the cartilaginous skeletal compartment is poorly addressed. To investigate the relation between chondrogenic differentiation, PGs and COX-enzymes we hypothesized that COX-1 and COX-2 have differential actions in the chondrogenic differentiation process of progenitor cells and that specific inhibition of their enzymatic activity leads to isotype-specific consequences on the outcome of the chondrogenic differentiation process.
Methods: ATDC5 cells were differentiated in the presence of different COX-1 (SC-560, Mofezolac) or COX-2 (NS398, Celecoxib) specific non-steroidal anti-inflammatory drugs (NSAIDs). COX-1 or COX-2 specificity of the NSAIDs and prostaglandin levels were determined by